Genetic variability in Modiolus modiolus populations from Newfoundland (Canada) revealed by protein electrophoresis with comparative notes on Mytilus spp.

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Abstract

Six polymorphic allozyme loci in horse mussel, Modiolus modiolus, and blue mussel, Mytilus spp., taken from mussel beds at four sites on the island of Newfoundland, Canada, were assayed using protein electrophoretic techniques on cellulose acetate. This is the first recorded study of biochemical genetic variability for North American populations of Modiolus modiolus. Significant heterozygote deficiencies compared to expected HWE were observed for Modiolus modiolus at five of the six loci. Population F-statistics revealed significant genetic heterogeneity among the four Modiolus populations. A significant positive relationship between inter-site pairwise F_{ST} values and inter-site geographic distance measured along the coastline was observed. UPGMA clustering of Nei's genetic distance values suggested a pattern of inter-site genetic similarity in parallel with the direction of flow of the Labrador Current, the main surface water circulation regime along the coast of Newfoundland. Comparison of the electrophoretic mobility of electromorphs in each of the two genera revealed that five of the six loci examined, Gpi, Pgm, Lap, Est, and Aap, showed evidence of alleles with electrophoretic mobilities unique to either Modiolus modiolus or to Mytilus spp. (M. edulis and M. trossulus). At the Gpi locus, all but two rare observed alleles were mutually exclusive between the two genera. The results from this study are used to advance testable hypotheses for further investigations with respect to suggested linkages among enzyme polymorphisms, environmental gradients, the natural population depth distributions of these related species, and the co-evolution of genetic variability in systematically related species in response to environmental heterogeneity.

Résumé

Les auteurs ont analysé six locus d'alloenzymes polymorphes de la moule modiole, Modiolus modiolus, et de la moule du genre Mytilus, prélevées dans des parcs à moules à quatre sites sur l'île de Terre-Neuve, au Canada. Ils ont utilisé des techniques d'électrophorèse des protéines sur acétate de cellulose. L'étude est la première à être publiée sur la variabilité génétique biochimique de populations nord-américaines de Modiolus modiolus. À cinq des six locus examinés pour cette espèce, les auteurs ont observé un déficit important en hétérozygotes, par rapport à ce qui serait attendu sous l'hypothèse d'un équilibre de Hardy-Weinberg (EHW). La statistique F de population a révélé une hétérogénéité génétique significative chez les quatre populations de moule modiole. Le long de la côte, on a noté une relation positive significative entre les valeurs F_{ST} pairées intersites et la distance géographique intersites. Le groupement par UPGMA des distances génétiques de Nei a révélé un profil de similarité génétique intersites qui correspond à la direction de l'écoulement du courant du Labrador, principal régime d'eau de surface le long de la côte de Terre-Neuve. Pour cinq des six locus examinés, soit Gpi, Pgm, Lap, Est et Aap, la mobilité électrophorétique des allèles était propre soit à Modiolus modiolus, soit au genre Mytilus (M. edulis et M. trossulus). Au locus Gpi, tous les allèles, sauf deux rarement observés, étaient exclusifs aux deux genres. Se basant sur les résultats de cette étude, les auteurs avancent des hypothèses vérifiables dans le cadre d'autres recherches ayant trait aux liens probables entre les polymorphismes enzymatiques, les gradients de conditions environnementales, les distributions en profondeur des populations naturelles de ces espèces

apparentées et la coévolution de la variabilité génétique chez des espèces régulièrement apparentées, compte tenu de la diversité des milieux.

Introduction

Four species of marine bivalve mussels are found naturally in coastal areas of Atlantic Canada. These are the horse mussel, Modiolus modiolus (Dillwyn), the ribbed mussel, Modiolus demissus (Dillwyn) and the conspecific blue mussels, Mytilus edulis (L.) and M. trossulus (Gould). In Atlantic Canada, Modiolus demissus is near the extreme northern limit of its range (Minor, 1950) and is very localized being largely restricted to the Gulf of St. Lawrence. Modiolus demissus is not found in Newfoundland. In contrast, the geographic distributions of the other three species are circumpolar and all are considered ubiquitous throughout Atlantic Canada and the northeast United States. Within Newfoundland, wild mussel beds in the inter-tidal and shallow sub-tidal zones are typically mixtures of the two Mytilus species, M. edulis and M. trossulus, living sympatrically (Bates and Innes, 1995; Penney and Hart, 1999). The depth range of Modiolus modiolus overlaps with the two Mytilus spp., but is typically centered in the sub-tidal zone and extends to much greater depths than Mytilus spp., sometimes exceeding 150 meters (Minor, 1950). In Newfoundland, the three mussel species are, collectively, the most predominant bivalve molluscs in benthic shoreline communities.

Genetic variation and population genetic structure of North American populations of the two Mytilus spp. have been extensively studied, using both electrophoretic techniques (Koehn et al. 1984; Varvio et al. 1988; McDonald et al. 1991; Sarver and Foltz, 1993; Bates and Innes, 1995; Penney and Hart, 1999) and, more recently, using DNA markers (Zouros et al. 1992; Heath et al. 1995; Comesaña et al. 1999). Despite its faunistic prominence in the littoral and sub-littoral zones, studies on the population genetics of Modiolus spp. are much more limited. Skibinski et al. (1980) investigated electrophoretic variation at thirteen loci in a Modiolus modiolus population from the United Kingdom while Koehn and Mitton (1972) and Koehn et al. (1973) reported on enzyme polymorphisms at three loci in the ribbed mussel, Modiolus demissus, from the Long Island area of the eastern United States. No genetic studies of North American Modiolus modiolus populations exist. The present study has two goals. The first is to provide information on genetic structure and variation within and among Modiolus modiolus populations in a mixed species zone in Newfoundland. The second is to compare electrophoretic variation between Modiolus modiolus and the two Mytilus spp., M. edulis and M. trossulus. The latter has potential use in future comparative studies of habitat and environmental preferences of the three species and offers further insight into their evolutionary and systematic relationships.

Methods

Samples of naturally occurring mixed species populations of Modiolus modiolus and Mytilus spp. were collected from four sites in Newfoundland, Canada: by hand-picking at an inter-tidal beach area on Ramea Island and by diver at depths of 3-10 meters from Shag Rocks, Comfort Cove, and Stock Cove (Figure 1). Samples were returned to the laboratory and held in tanks at ambient water temperatures until utilized. Hepatopancreas tissue was excised, lyophilized, and stored at 5° C for later analysis. A small amount of freeze-dried material was chopped to a fine powder and ground with Tris HCL pH 8.0 buffer with 20% glycerol. Eight loci, initially selected because they were previously reported to be polymorphic in Mytilus spp. (MacDonald and Koehn, 1988; Sarver and Foltz, 1993; Bates and Innes, 1995), were screened in test runs to determine resolution, legibility, and scorability. These were mannose phosphate isomerase (Mpi, EC 5.3.1.8), aminopeptidase-I (Lap, EC 3.4.11.-), phosphoglucomutase (Pgm, EC 2.7.5.1), glucose-6-phosphate isomerase (Gpi, EC 5.3.1.9), aminopeptidase (Ap, EC 3.4.-.-), peptidase II (Aap, EC 3.4.-.-), esterase (Est, 3.1.1.1), and octopine dehydrogenase (Odh, EC 1.5.1.11). Cellulose acetate electrophoresis and staining were carried out according to procedures described by Hebert and Beaton (1989), with the following modifications: Lap, Aap, and Odh were run with Tris glycine pH 8.6 buffer; Aap staining used the method of Young et al. (1979); and Odh staining was adapted for cellulose acetete from the method of Dando et al. (1981). We were unable to resolve Mpi or Odh with freeze-dried tissue from Modiolus modiolus on the cellulose acetate system, although both could be resolved quite well for Mytilus spp.

Individual Mytilus spp. in the present study were classified to their respective species based on their Mpi genotype (Varvio et al. 1988; McDonald et al. 1991). Since no previous references designating and numbering Modiolus modiolus electromorphs exist for North American populations, allelic variation for Mytilus spp. was designated first using allele nomenclature similar to that employed for Mytilus spp. by previous authors (Koehn et al. 1984; McDonald and Koehn 1988; Penney and Hart, 1999). Alleles found in Modiolus modiolus with the same electrophoretic mobility compared to those of Mytilus spp. were given the same designation. Those alleles with clearly different mobility were designated relationally to known Mytilus alleles. For Est, electromorphs found in the Mytilus spp. individuals (and those of a test sample of known M. edulis and M. trossulus) could not be positively equated to those reported previously for Est-D in Newfoundland populations using starch gel electrophoresis with other substrates (Varvio et al. 1988; Bates and Innes, 1995). This being the case and, since the esterases are known to be a complex, multi-locus system (Richardson et al. 1986), it is possible the esterase detected by our methodology may be a different locus than that reported in the latter two studies. Therefore, we simply designated the Est alleles in order of observed mobility starting with 1 as the slowest electromorph.

Analysis of allele frequencies, Wright's F statistics with jackknifed estimators (Weir and Cockerham, 1984) and associated probability testing were carried out using FSTAT version 1.2 (Goudet, 1995). Other descriptive statistics, mean heterozygosities, heterozygote deficiencies ($1-H_o/H_e$), χ^2 and G-statistic significance tests, and Nei's genetic distances were calculated using G-

Stat, version 3.1 (Siegismund, 1995), with genetic distance output files processed into UPGMA cluster dendrograms using PHYLIP, version 3.57c (Felsenstein, 1995). The R x C χ^2 contingency tests of interspecific heterogeneity between allele frequencies of Modiolus modiolus and Mytilus edulis were calculated using SAS (SAS Institute Inc., 1985).

Results

Electromorph patterns

Mytilus spp. samples from three of the four sites were found to be mixtures of both M. edulis and M. trossulus. Shag Rocks was the only site where M. trossulus was not present. The incidence of M. trossulus at the other three sites was low relative to M. edulis: 7.7% at Ramea, 10.6% at Comfort Cove, and 15.2% at Stock Cove. These low numbers (13 individuals in total) were insufficient to support interspecific comparative analyses of M. trossulus with the other two species. Therefore, data from published references for other Newfoundland Mytilus spp. populations (Bates and Innes, 1995; Penney and Hart, 1999) were included for comparative purposes in Tables 1 and 2. Table 1 summarizes data on the number of alleles found for Modiolus modiolus and Mytilus spp. for all loci over the four sample locations and incorporates known alleles for other Newfoundland Mytilus spp. populations. All 6 loci were polymorphic (at the 95% criterion level) at all sites, with the exception of Est which was virtually monomorphic for both Modiolus modiolus and Mytilus edulis at Stock Cove (Table 2). In Modiolus modiolus, the number of alleles per locus ranged from a low of 4 at the Est locus to a high of 12 at Lap. The mean number of alleles per locus as well as the number of alleles at each individual locus in Mytilus spp. were approximately the same as Modiolus modiolus (Table 1), differing by no more than one allele at any locus with the exception of Lap which had only 8 alleles in Mytilus spp. compared to 12 in Modiolus modiolus.

Both Modiolus modiolus and Mytilus spp. have many alleles of similar electrophoretic mobility on cellulose acetate. For the Aap, Ap, and Est loci, alleles unique to either species (Table 1) tend to be relatively rare alleles (Table 2). However, at the Lap, Pgm, and particularly at the Gpi locus, alleles common in Modiolus modiolus may be regarded as unique for the species since they were completely absent in either of the Mytilus spp. These include Lap^{99}, Lap^{97}, Lap^{95}, Lap^{93}, Lap^{91}, and Lap^{89}, as well as Pgm^{109} and Pgm^{104}. For Gpi, at the four sample sites, the allele frequency distribution of Modiolus modiolus was completely non-overlapping with Mytilus edulis (and with all Newfoundland populations of M. trossulus).Only a few relatively rare alleles at the remaining loci are unique to Modiolus modiolus. At the Aap, Ap, and Est loci in particular, many electromorphs of similar electrophoretic mobility exist among the three species and allele frequencies of Modiolus modiolus and Mytilus edulis overlap considerably at each of the four sites. R x C contingency χ^2 tests of interspecific allele frequency heterogeneity between Modiolus modiolus and Mytilus edulis were significantly different at all loci when pooled over all populations (Table 3) as well as for most site x locus combinations individually. Exceptions to

this included the Aap locus at which interspecific comparisons were significantly different only at Ramea and Comfort Cove, and the Est locus which was significantly different only at Ramea.

Modiolus modiolus population genetic structure

Population observed and HWE-expected heterozygosities for all site x locus combinations, as well as the means for each site over all loci, are given in Table 4. Significance testing of Selander's D values using the G test statistic for pooled alleles (Siegismund, 1995) revealed significant heterozygote deficiencies within most populations at each site for all loci except Est (Table 5). Population F-statistics at individual loci and jackknifed estimators of the means over all loci were generally statistically significant (Table 6). F_{IS} and F_{IT} values were significant at all individual loci (and means over all loci pooled) indicating significant non-random departures from Hardy Weinberg Equilibrium both within sites as well as over all sites pooled. F_{ST} values were significant at all individual loci (as well as the means over all loci pooled) except Aap and Ap, indicating significant genetic differentiation between the four sites at all except the latter two loci.

Matrices of genetic similarity based on Nei's genetic distance and identity estimates are given in Table 7. A marginally significant relationship was found between inter-site geographic distance measured along the coastline and inter-site pairwise F_{ST} estimates of genetic heterogeneity (SAS GLM, $r^2=0.63$, $p< 0.05$). The clustering UPGMA dendrogram of Nei's distance values (Figure 2) is suggestive of a possible clinal, north-south geographic relationship. All four sites show a relational pattern of increasing genetic distance in accordance with their geographic location along the coastline. Shag Rocks and Stock Cove are most similar of the four sites. Ramea originates from the same branch while Comfort Cove, the most northerly of the four sites, is most dissimilar of all. The existence of an allelic cline (on a north-south axis in parallel with the Labrador Current, the predominant surface water circulation regime in coastal Newfoundland) could not be confirmed since regression analyses of the frequency of each of the major alleles per locus as a function of geographic distance along the north-south axis were not significant (SAS GLM, $p> 0.05$). With only four sites studied, the possible existence of a clinal relationship in allele frequencies among Modiolus modiolus populations remains speculative.

Discussion

The present study provides the first recorded analysis of electrophoretic variation and genetic structure in North American populations of Modiolus modiolus. Only one other record of an electrophoretic investigation exists for Modiolus modiolus, and that is for a European population by Skibinski et al. (1980) who assayed allele frequencies at 13 loci for a sub-littoral population from the Isle of Man in the Irish Sea. In their investigation, Skibinski et al. (1980) found 7 of 13 loci to be polymorphic. Four of these loci, Ap, Pgm, Gpi, and Lap (=Lap-2 of Skibinski et al.,

1980) (Koehn et al. 1984)) were also assayed in our work. Compared to the Isle of Man population, Newfoundland Modiolus populations had more alleles per locus, particularly at Lap and Gpi, but observed heterozygosities at the four loci in common between the two studies were remarkably similar in the two populations. For both the Isle of Man and Newfoundland populations, Ap had the lowest observed heterozygosity, Lap and Pgm were intermediate, and Gpi was highest. The mean observed heterozygosity over all four loci for Isle of Man (0.462) was also within the range observed for the four Newfoundland populations (0.38-0.55). Unlike the Newfoundland populations in the present study, Skibinski et al. (1980) did not record a significant heterozygote deficiency for M. modiolus at these same loci.

When compared with nearby Mytilus edulis populations in England, the general patterns of polymorphism in Modiolus modiolus at both the Gpi locus (allelic frequencies non-overlapping with M. edulis, M. modiolus with lower electrophoretic mobility), and at the Pgm locus (species-specific alleles of alternating relative electrophoretic mobility), are similar to the situation found for Newfoundland populations in our work. However, Skibinski et al. (1980) found most alleles at the Lap locus to be common to both species, and the allelic frequency distributions to be quite similar. This is in contrast with our finding that the major Lap alleles of both species are electrophoretically different between species although their relative electrophoretic mobilities closely approximate each other. The observed pattern of polymorphism we found at the Lap locus for Newfoundland Modiolus modiolus in relation to Mytilus spp. (species-specific alleles with alternating relative mobility) is, however, similar to that reported for its congener, Modiolus demissus, in relation to sympatric Mytilus edulis from Long Island, New York (Koehn and Mitton, 1972). The situation reported for Ap in the Isle of Man population of Modiolus modiolus is also very different from Newfoundland populations. Where Skibinski et al. (1980) reported non-overlapping allelic frequencies with nearby M. edulis populations, we observed overlapping allelic frequencies with M. edulis at all four sites. However, in terms of relative intra-specific frequency, the distributions of Ap alleles in Newfoundland M. edulis and Modiolus modiolus were significantly different.

In the present study, electrophoretic mobilities at the Gpi locus in Modiolus modiolus were virtually completely distinct from Mytilus spp. The only electrophoretic overlap between Modiolus modiolus and Mytilus spp. at the Gpi locus in Newfoundland populations was for two relatively rare alleles, Gpi^{79} and Gpi^{75}. Although these two alleles together reached a maximum frequency of ~ 8% in Modiolus modiolus, in an extensive sampling of Newfoundland Mytilus spp. populations Penney and Hart (1999) recorded a maximum combined frequency of <2% at any site for these alleles in M. edulis, and zero in M. trossulus. Newfoundland populations of Modiolus modiolus also have some alleles at relatively high frequency at both the Pgm and Lap loci which are not found among Mytilus spp. At other loci, most electromorphs found in Modiolus modiolus were of similar electrophoretic mobility to electromorphs in Mytilus spp. Although the four sites surveyed in the present work provided comparative data for only Modiolus modiolus and Mytilus edulis (M. trossulus numbers were very low at the sampling sites), reference to existing allozyme analyses for M. trossulus from other Newfoundland sites (Bates and Innes, 1995; Penney and Hart, 1999) indicate the foregoing conclusions related to M.

edulis are valid for M. trossulus as well.

Significant genetic heterogeneity in M. modiolus among sites, as evidenced by population F statistics, was observed at all loci except Aap and Ap. In most cases, the inter-population heterogeneity may be ascribed to variation in the frequency of the most common alleles, although the occurrence of some relatively rare alleles was at times uneven among populations. The cause or causes of the observed inter-population genetic heterogeneity among Newfoundland Modiolus modiolus populations may be due to several factors, although the Wahlund effect must be considered likely to be at least partially responsible, as is the case for genetic heterogeneity observed in Newfoundland Mytilus spp. populations (Raymond, 1997).

Analysis of the inter-population genetic similarity matrix by UPGMA produced a dendrogram wherein the order of the four sites paralleled the north-south direction of the Labrador Current which is the major surface water flow along the Newfoundland coastline (Colbourne et al. 1997). Under the influence of the Labrador Current, genetic exchange among adjacent populations would likely be non-random with active genetic exchange from north to south. The mean north-south water transport velocity in the Labrador Current along the northeast coast of Newfoundland is in the order of 0.05-0.1 meters sec^{-1} (Colbourne et al., 1997). With an approximate pelagic larval development time of 30 days estimated from prevailing water temperatures and development times for Modiolus modiolus (de Schweinitz and Lutz, 1976), horizontal transport of Modiolus larvae is not likely to exceed 260-280 km. Of the four sample locations, the only inter-site geographic distance within this range is that between Comfort Cove and Stock Cove. Hence, most of the sites are sufficiently far apart geographically to be considered effectively isolated. This conclusion is reinforced by the observed marginally significant positive relationship between the inter-site pairwise F_{ST} values and geographic distance. Taking into consideration the limited sampling of just four sites in the present study, the observed pattern of among-site genetic heterogeneity is suggestive of the isolation by distance model of gene flow (see Slatkin, 1993, for review), in this case mediated by the prevailing oceanographic water circulation patterns of the Labrador Current around the coast of Newfoundland. However, this interpretation and that of the existence of allelic clines must be considered highly speculative with the present limited sampling of populations. Further population surveys including more sample sites are required to provide a more definitive testing of this hypothesis.

The existence of environmentally-mediated genetic heterogeneity in such a geographically widespread species as Modiolus modiolus may be considered highly probable. In this regard, the comparative electrophoretic mobilities of alleles at the Gpi locus in Modiolus modiolus and Mytilus spp. is particularly interesting. Latitudinal clines which parallel thermal gradients have been observed at the Gpi locus in a variety of finfish and invertebrate species (see Hall 1985) suggesting a possible relationship between allelic variability at the Gpi locus and temperature. Both Hall (1985) and Hoffmann (1984) have produced evidence that the enzyme products of individuals with electrophoretically faster Gpi alleles at this locus exhibit superior biochemical activity at higher temperatures in mussels and anemones respectively. More recently, Penney and Hart (2002) produced evidence of genotype-dependent survival selection favoring survival of Gpi

genotypes with electrophoretically slower alleles in comparison to genotypes with electrophoretically faster alleles in sub-tidally cultured Mytilus spp. in Newfoundland. These cultured populations are not subjected to the relatively high air temperatures experienced during emersion by their natural inter-tidal wild counterparts. Thus, we may speculate the natural mainly inter-tidal depth distribution of Mytilus spp. versus the mainly sub-tidal depth distribution of Modiolus modiolus may be a consequence of biochemical adaptation to different environmental temperature ranges by these species. Or, in other words, the virtual exclusion of M. modiolus from the intertidal zone in favor of Mytilus spp. may be a consequence of differing relative metabolic efficiencies of the enzyme products produced by their respective Gpi loci. The extensive micro and macro-geographic overlap between the faunistically prominent Modiolus modiolus and Mytilus spp. in the northern hemisphere makes this taxonomic group an excellent candidate for future investigations to test this hypothesis and others concerning the co-evolution of genetic variability in systematically related species in response to environmental heterogeneity. The results of the present work comparing the enzyme polymorphisms of sympatric Modiolus modiolus and Mytilus spp. populations in Newfoundland as well as the genetic structure of M. modiolus populations provide the basis for more detailed investigations in this area.

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Tables

Table 1. Number of alleles at each of six loci and mean number of alleles per locus in Modiolus modiolus and Mytilus spp. The listing of alleles unique to each species includes those identified from the present work as well as known alleles previously reported from other sources for Mytilus spp. in Newfoundland (Bates and Innes, 1995; Penney and Hart, 1999).

Locus	# of alleles (M. modiolus)	Alleles unique to M. modiolus	# of alleles[1] (Mytilus spp.)	Alleles unique to Mytilus spp.
Lap	12	Lap^{99}, Lap^{97} Lap^{95}, Lap^{93} Lap^{91}, Lap^{89}	8	Lap^{102}
Pgm	6	Pgm^{109}, Pgm^{104}	6	Pgm^{118}, Pgm^{93} Pgm^{89}
Gpi	11	All except Gpi^{79}, Gpi^{75}	12	All except Gpi^{79}, Gpi^{75}
Aap	7	Aap^{85}	6	Aap^{115}
Ap	7	None	7	None
Est	4	None	5	Est^{5}
Mean	7.83		7.33	

[1] includes Lap^{102}, Lap^{88}, Gpi^{79} and Gpi^{75} reported for M. edulis by Penney and Hart (1999)

Table 2. Allele frequencies for 6 loci at each of four sites for Modiolus modiolus (MM) and Mytilus edulis (ME), with comparative frequencies of M. trossulus (MT) from other published references for pooled Newfoundland populations (Lap, Gpi, and Pgm from Penney and Hart, 1999; Ap from Bates and Innes, 1995). (N)= sample size

	Ramea Island		**Shag Rocks**		**Stock Cove**		**Comfort Cove**		
Locus/ allele	MM	ME	MM	ME	MM	ME	MM	ME	MT
Lap									
(N)	49	36	67	22	52	40	32	42	
100	0.010	0.014	-	-	-	-	-	0.012	0.007
99	0.051	-	0.022	-	0.010	-	-	-	-
98	0.020	0.556	0.090	0.545	-	0.650	0.063	0.610	0.176
97	0.051	-	0.060	-	0.010	-	0.016	-	-
96	0.265	0.361	0.112	0.409	0.163	0.250	0.063	0.317	0.398
95	0.347	-	0.455	-	0.548	-	0.547	-	-
94	0.061	0.069	0.045	0.045	0.010	0.063	0.078	0.061	0.324
93	0.041	-	0.022	-	0.087	-	0.125	-	-
92	0.010	-	0.037	-	0.019	0.013	0.000	-	0.081
91	0.112	-	0.112	-	0.048	-	0.063	-	-
90	0.010	-	0.037	-	0.038	-	0.016	-	0.014
89[1]	0.020	-	0.007	-	0.067	-	0.032	-	-
Pgm									
(N)	49	36	67	22	52	40	32	42	
114[2]	0.031	0.097	0.015	0.091	0.029	0.112	0.016	0.048	0.107
111	0.061	0.056	0.007	0.068	0.019	0.038	-	0.095	0.464
109	0.133	-	0.224	-	0.260	-	0.406	-	-
106	-	0.181	0.030	0.182	0.019	0.138	0.031	0.083	0.220
104	0.714	-	0.716	-	0.644	-	0.547	-	-
100	0.061	0.625	0.007	0.636	0.029	0.637	-	0.750	0.186
93[3]	-	0.042	-	0.023	-	0.075	-	0.024	0.022

	Ramea Island		Shag Rocks		Stock Cove		Comfort Cove		
Locus/ allele	MM	ME	MM	ME	MM	ME	MM	ME	MT
Est									
(N)	49	36	67	24	52	27	32	41	
1	-	0.014	-	-	-	0.019	0.094	0.134	
2	0.041	0.056	0.060	0.042	-	-	0.016	0.085	
3	0.806	0.903	0.888	0.938	0.990	0.981	0.844	0.707	
4[4]	0.153	0.028	0.051	0.021	0.010	-	0.047	0.073	
Gpi									
(N)	49	36	67	22	52	40	32	42	
110	-	-	-	-	-	0.054	-	0.061	0.009
107	-	0.147	-	0.028	-	0.189	-	0.134	0.025
102	-	0.118	-	0.139	-	0.108	-	0.195	0.058
100	-	0.250	-	0.250	-	0.284	-	0.122	0.147
98	-	0.221	-	0.306	-	0.162	-	0.232	0.359
96	-	0.088	-	0.111	-	0.041	-	0.134	0.232
93	-	0.103	-	0.167	-	0.054	-	0.085	0.094
89	-	0.074	-	-	-	0.054	-	0.037	0.060
86	-	-	-	-	-	0.054	-	-	0.011
83	-	-	-	-	-	-	-	-	0.004
79	0.020	-	0.007	-	0.010	-	-	-	-
75	0.071	-	0.082	-	0.010	-	0.016	-	-
72	0.163	-	0.157	-	0.154	-	0.094	-	-
69	0.204	-	0.119	-	0.163	-	0.094	-	-
66	0.133	-	0.261	-	0.260	-	0.281	-	-
62	0.265	-	0.082	-	0.173	-	0.281	-	-
59	0.061	-	0.119	-	0.087	-	0.063	-	-
56	0.051	-	0.119	-	0.077	-	0.078	-	-
53[5]	0.030	-	0.052	-	0.067	-	0.094	-	-

	Ramea Island		Shag Rocks		Stock Cove		Comfort Cove		
Locus/ allele	MM	ME	MM	ME	MM	ME	MM	ME	MT
Ap									
(N)	49	36	67	24	52	27	32	41	
108	0.010	0.097	-	-	-	0.074	-	0.073	-
105	0.082	0.639	0.082	0.500	0.058	0.500	0.141	0.500	0.285
100	0.735	0.181	0.769	0.208	0.702	0.259	0.734	0.195	0.469
95	0.061	0.056	0.030	0.188	0.096	0.074	0.016	0.110	0.098
90	0.082	0.028	0.104	0.083	0.135	0.056	0.094	0.098	0.125
85[6]	0.031	-	0.014	0.021	0.010	0.037	0.016	0.024	-
Aap									
(N)	49	36	67	24	52	27	32	41	
110[7]	0.041	-	-	-	-	0.019	-	0.036	
105	0.041	0.014	0.104	0.021	0.067	0.019	0.109	0.024	
100	0.847	0.889	0.769	0.896	0.760	0.833	0.703	0.890	
95	0.071	0.056	0.082	0.063	0.058	0.111	0.094	0.024	
90	-	-	0.022	-	0.048	-	0.063	-	
85	-	-	-	-	-	-	0.016	-	
80	-	0.042	0.022	0.021	0.067	0.019	0.016	0.024	

[1] includes rare allele Lap^{88}; [2] includes rare allele Pgm^{118}; [3] includes rare allelePgm^{89}; [4] includes rare allele Est^{5}; [5] includes rare alleles Gpi^{50} and Gpi^{47}; [6] includes rare allele Ap^{80}; [7] includes rare allele Aap^{115}

Table 3. Probability matrix for R x C Contingency χ^2 tests of interspecific allele frequency heterogeneity for 5 loci in four mixed populations of Modiolus modiolus and Mytilus edulis and for all populations pooled. Gpi is omitted since the interspecific allele frequencies are non-overlapping. (Degrees of freedom are adjusted as required to ensure statistical validity)

Locus	Ramea	Shag Rocks	Stock Cove	Comfort Cove	All Pops. Pooled
Lap	<0.001 (df=8)	<0.001 (df=8)	<0.001 (df=8)	<0.001 (df=7)	<0.001 (df=8)
Pgm	<0.001 (df=5)	<0.001 (df=5)	<0.001 (df=5)	<0.001 (df=5)	<0.001 (df=5)
Aap	<0.05 (df=3)	n.s.	n.s.	<0.02 (df=3)	<0.003 (df=3)
Ap	<0.001 (df=4)	<0.001 (df=4)	<0.001 (df=4)	<0.001 (df=4)	<0.001 (df=4)
Est	<0.02 (df=2)	n.s.	n.s.	n.s.	<0.02 (df=3)

Table 4. Observed (H_o) and Hardy-Weinberg Equilibrium expected (H_e) heterozygosities at six loci (and means over loci) for Modiolus modiolus in four sampled populations from Newfoundland. ( ) = standard errors

Locus	Ramea		Shag Rocks		Stock Cove		Comfort Cove	
	H_o	H_e	H_o	H_e	H_o	H_e	H_o	H_e
Lap	0.45	0.78	0.57	0.75	0.66	0.33	0.65	0.35
Pgm	0.43	0.46	0.33	0.44	0.42	0.52	0.35	0.56
Gpi	0.71	0.83	0.69	0.85	0.61	0.84	0.76	0.82
Aap	0.12	0.27	0.33	0.39	0.29	0.41	0.34	0.48
Ap	0.29	0.44	0.30	0.39	0.35	0.46	0.34	0.43
Est	0.31	0.33	0.16	0.21	0.02	0.02	0.22	0.28
Mean	0.38 (0.08)	0.53 (0.10)	0.40 (0.08)	0.51 (0.10)	0.36 (0.08)	0.49 (0.12)	0.41 (0.08)	0.55 (0.08)

Table 5. Selander's D ($1-(H_o/H_e)$) values and G-tests of deviation from Hardy-Weinberg Equilibrium for each of six loci, using pooled alleles, for four populations of Modiolus modiolus. Numbers without asterisks are not significant at p=0.05.

Locus	Ramea	Shag Rocks	Stock Cove	Comfort Cove
Lap	0.43**	0.24*	0.33*	0.35**
Pgm	0.08	0.25*	0.18	0.37**
Gpi	0.14*	0.19*	0.28**	0.06
Aap	0.55**	0.16	0.29*	0.28**
Ap	0.35**	0.24*	0.23	0.20*
Est	0.06	0.20	0.01	0.21

* = $p<0.05$; ** = $p<0.01$

Table 6. Wright's F statistics for six individual loci and jackknifed estimators of the means over all loci for all sampled populations of Modiolus modiolus.

Locus	F_{IS}	F_{IT}	F_{ST}
Lap	0.333**	0.345**	0.017**
Pgm	0.224**	0.238**	0.017*
Gpi	0.198**	0.208**	0.013**
Aap	0.290**	0.292**	0.001
Ap	0.269**	0.266**	-0.004
Est	0.146*	0.181**	0.043**
Mean	0.254**	0.265**	0.014**

* = $p<0.05$; ** = $p<0.01$

Table 7. Matrix of genetic similarity for Modiolus modiolus populations based on Nei's genetic distance (below diagonal) and identity values (above diagonal).

Population	Ramea	Shag Rocks	Stock Cove	Comfort Cove
Ramea	---	0.9639	0.9581	0.9277
Shag Rocks	0.0368	---	0.9779	0.9568
Stock Cove	0.0428	0.0226	---	0.9637
Comfort Cove	0.0750	0.0442	0.0370	---

Figures

Figure 1. Map of the island of Newfoundland showing the four sampling locations. Arrows indicate the path of the Labrador Current, the major surface water circulation pattern (adapted from Colbourne et al., 1997).

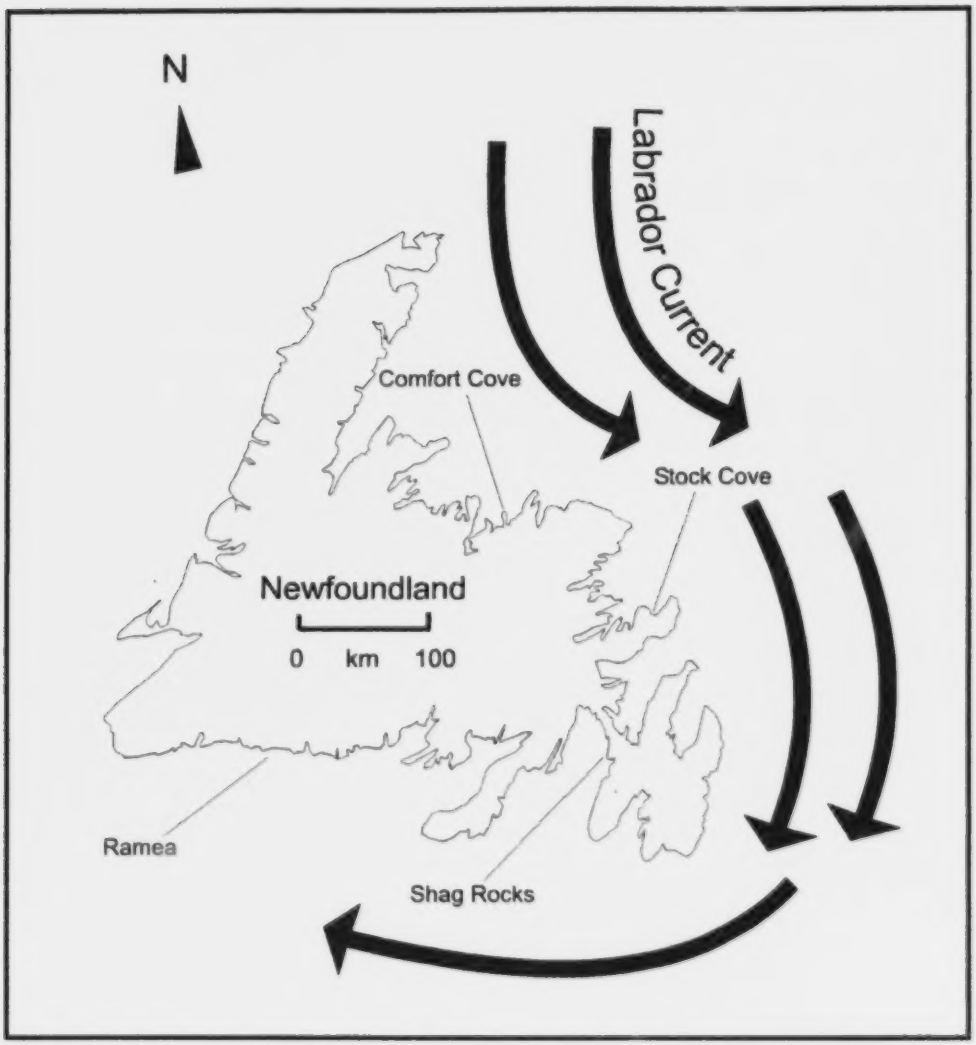

Figure 2. UPGMA clustering dendrogram based on Nei's genetic distance values from the four sampled Modiolus modiolus populations.